SELF-INCOMPATIBILITY IN EUCALYPTUS

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Eucalypts are generally preferentially outcrossing (Pryor 1976; Griffin et al 1987), with relatively high outcrossing rates (e.g. 0.69-0.84 Moran and Bell 1983) maintained by varying degrees of self-fertility (Pryor 1957, 1976; Hodgson 1976b; Griffin et al 1987), aided by protandry (Pryor 1976) and reinforced by selection against the products of self-fertilization in later stages of the life cycle (see Potts et al 1987). Most species exhibit a marked reduction in seed yield following self-pollination compared to outcrossing, although within species there is considerable variation in the level of self-fertility (Pryor 1957, 1976; Table 1). In most of the species examined in detail to date, the majority of individuals are partially self-fertile, but individuals range from fully self-incompatible to fully self-fertile (Table 1)[1]. Postmating barriers to self-fertilization are thus rarely complete, and Eldridge (1976) notes of the genus that "persistent attempts at artificial self-pollination have been successful to some degree on almost every tree tested". Griffin et al (1987) later noted that with only one possible exception, all the E. regnans trees they had investigated were self-fertile to some degree, and Pryor (1957) states that of some dozens of individuals examined of nine species only one tree of E. bicostata was found to be fully self-incompatible. Early reports of self-sterile species (Krung and Alves 1949) have not been substantiated (e.g. E. grandis - Hodgson 1976a,b), although Eldridge (1976) did note the failure of self-pollination of E. deglupta while outcrossing was successful.

1 Male steriles, although rare have also been noted (pryor 1976); the female component is normal and thus the plants are effectively unisexual and obligate outbreeders.

Considerable variability in the success of replicate self-pollinations has been reported (Tibbits 1988), and clearly adequate controls and several independent replications of a cross are required before a plant can be reliably classified as fully self- incompatible.

Self-incompatibility in E. morrisbyi

Recent pollination studies have shown that the frequency of fully self-incompatible individuals in the rare Tasmanian endemic, E. morrisbyi, is one of highest recorded in the genus to date. While no more than 20% of individuals in species such as E. regnans, E. grandis, E. nitens, E. globulus and E. gunnii were classified as self-incompatible, over 80% of the E. morrisbyi trees examined were self-incompatible (SI > 95%; Table 1).

Two approaches were used to estimate the level of self-incompatibility (SI). Control crosses were undertaken with flowers emasculated and self and outcross pollen manually applied (e.g. assisted crosses; Table 2) or alternatively unopened flowers were simply bagged with flies (unassisted crosses; see Pryor 1976; Eldridge and Griffin 1983) and open-pollinated flowers used as the control. A plant was classified as self-incompatible when the percentage reduction in seed yield after selfing was over 95% less than the yield following outcrossing or from open-pollination. Comparable results were obtained from unassisted and assisted self-pollinations (e.g. Table 2), but the poorer seed yield following open- pollination compared to controlled outcrossing (Table 2) resulted in a more conservative estimate of the level of self-incompatibility using the latter method.

A 4 x 4 diallel mating was undertaken in 1986 and many of the crosses were repeated in 1987. Seed was obtained from all outcrosses in at least one year indicating that all outcrosses are fully compatible (Table 2). The better seed yield in 1987 was mainly a result of improved pollination techniques and all of the outcrosses which failed in 1986 were successful when repeated. In

contrast, in both years no or trivial amounts (SI > 99%) of selfed seed was obtained from the 4 trees. Self-pollinations were replicated from 4 to 7 times over the two years which strongly indicates that two of the trees (MOR2 and MOR5) are fully self-incompatible and the other two more or less so. Of the nineteen trees tested by unassisted selfing in 1987 (including trees in Table 2; 2 replicated bags/cross), eleven produced no selfed seed (SI = 100%), five yielded less than 96% that of the open, two trees were partially self-incompatible (SI = 73 and 53 %) and only one individual was fully self-fertile (SI < 10%). A twentieth tree tested aborted all flowers regardless of pollination treatment. Open pollination was successful on all other trees (mean = 1.39; range 0.12 - 2.55 seed/flower). However, the yield of seed following open pollination was only 7% of that following controlled outcrossing (1987 mean = 20.5; range 15.7 - 23.2 seed/flower). This low seed yield is mainly due to much lower seed set per capsule following open-pollination. Other experiments where flowers were emasculated and either enclosed or left open, indicated that seed set in identical pollinations was increased by enclosing flowers in pollination bags, probably due to increasing temperature and protection. However, this bagging effect alone was insufficient to account for the reduced seed set in open pollination, suggesting that natural seed set in E. morrisbyi may be pollinator limited (c.f. E. regnans - Griffin et al 1987).

Mechanism of self-incompatibility

Pryor (1957, 1976) indicates there is some evidence for a genetic controlled self-incompatibility system in some species of Eucalyptus. However, as noted by Griffin et al (1987), this was inferred from observations of self-fertility, the mechanistic basis of which may vary, and they consider that there is no conclusive evidence for self- incompatibility reactions in Eucalyptus. Reduced seed set following self- pollination may result from the operation of gametophytic or sporophytic self-incompatibility systems or inbreeding depression arising from the embryonic expression of recessive lethal genes (de Nettancourt 1977; Seavey

and Bawa 1986). However, while de Nettancourt (1977) restricts the definition of self-incompatibility to prezygotic mechanisms mainly operative in the stigma or style, ovarian and even postzygotic inhibition are noted, and Seavey and Bawa (1986) consider that such late-acting self- incompatibility may be quite common.

The genetic basis of variation in self-incompatibility, and the details of mechanisms controlling preferential outcrossing and self- incompatibility in the Eucalytpus are at present unclear. However, in E. regnans, the species studied in most detail to date, preferential outcrossing appears to be mainly controlled by postzygotic mechanisms (Griffin et al 1987). No consistent differences in pollen tube growth following self- and cross-pollination have been observed in the styles of E. regnans (Griffin et al 1987), E. globulus (unpubl. data) nor E. morrisbyi (unpubl. data). Selfed pollen has germinated and grown past the base of the style in self-incompatible trees of E. morrisbyi. Stigmatic inhibition can clearly be discounted and it is unlikely that a stylar self-incompatibility system is operative in any of these species, although the rate of pollen tube growth requires further study. The absence of inhibition in the style is also consistent with the presence of hollow or partly hollow styles in Eucalyptus (Boland and Sedgley 1986); the styles are hollow in most species where the incompatibility reaction occurs in the ovary (de Nettancourt 1977). While inbreeding depression could account for reduced self seed set in other species of Eucalyptus, following the criteria given by Seavey and Bawa (1986), the zero or near zero self seed set in most individuals of E. morrisbyi would strongly suggest that active self-rejection is occurring and that some sort of late-acting self- incompatibilty mechanism is operative. Further work on this species could provide the first positive identification of a self-incompatibility system in Eucalyptus and E. morrisbyi would be a clear example on which to ellucidate the genetic and physiological nature of the self-incompatibility reaction.

Acknowledgements

B.M.P. acknowledges receipt of a Australian Government National Research Fellowship.

References

Boland, D. J. and Sedgley, M. 1986. Stigma and style morphology in relation to taxonomy and breeding systems in Eucalyptus and Angophora (Myrtaceae). Aust. J. Bot. **34**: 569-84.
de Nettancourt, D. 1977. 'Incompatibility in Angiosperms'. (Springer- Verlag, Germany.)
Eldridge, K. G. 1976. Breeding systems, variation and genetic improvement of tropical eucalypts. In ' Tropical Trees: Variation, Breeding and Conservation'. Eds. J. Burley and B.T. Styles. pp. 101- 108. (Academic Press, London.)
Eldridge K. G. and Griffin , A. R. 1983. Selfing effects in Eucalyptus regnans. Silvae Genetica **32**: 216-221.
Griffin, A. R., Moran, G. F. and Fripp, Y. 1986. Preferential outcrossing in Eucalyptus regnans. Aust. J. Bot. **35**: 465-475.
Hodgson, L. M. 1976a. Some aspects of flowering and reproductive behavior in Eucalyptus grandis (Hill) Maiden at J. D. M. Keet forest research station. 2. The fruit, seed, seedlings, self fertility, percent selfing and inbreeding effects. S.A. For. J. **98**: 32-43.
Hodgson, L. M. 1976b. Some aspects of flowering and reproductive behavior in Eucalyptus grandis (Hill) Maiden at J. D. M. Keet forest research station. 111. Relative yield, breeding systems, barriers to selfing and general conclusions. S.A. For. J. **99**: 53-58.
Krug, C. A. and Alves, A. S. 1949. Eucalyptus improvement. J. Hered. **40**: 133-9.
Moran, G. F. and Bell, J. C. 1983. Eucalypts. In 'Isozymes in Plant Genetics and Breeding: Part B' Eds. S.D. Tankley and T. J. Orton, pp. 423-441. (Elsevier Science Publishers, Amsterdam.)
Potts, B. M., Potts, W. C. and Cauvin, B. 1987. Inbreeding and interspecific hybridisation in Eucalyptus gunnii. Silvae Genetica **36**: 194-199.
Potts, B. M. and Cauvin, B. 1988. Inbreeding and interspecific hybridisation of Eucalyptus. In " Proceedings of the Internalional Forestry Conference for the Australian Bicentenary. (Albury-Wodonga, Australia.)
Pryor, L. D. 1957. Selecting and breeding for cold resistance in Eucalyptus. Silvae Genetica **6**: 98-109 .
Pryor, L. D. 1976. 'Biology of the eucalypts'. (Arnold, London.)
Seavey, S. R. and Bawa, K.S. 1986. Late-acting self-incompatibility in Angiosperms. Bot. Rev. **52**: 195-219.
Tibbits, W. 1988. Controlled Pollination Studies with Eucalyptus nitens (Deane & Maiden) Maiden. J. Forestry, (in press).

Table 1 The level of self-incompatibility in some eucalypt species.

The overall level of self-incompatibility (SI) is measured as the percentage reduction in seed yield (per flower crossed or capsule harvested) following controlled self-pollination compared to outcrossing and is based on grand means for each pollination type. The classification of individuals was based on either the assignment reported or the calculated level of self-incompatibility (fully self-incompatible SI> 95%, partially self-incompatible 5%< SI ≤ 90% or fully self-compatible SI <5%). Pollen from a different population was used as the outcross control in the case of *E. nitens*, *E. ovata*, *E. globulus* and *E. gunnii*. ([1]further testing required)

Species	level of SI (%)		Percentage of trees			N	Source
	seed/flower	seed/capsule	fully SI	partially SI	fully self-fertile		
E. regnans	48 69	47 41	7 0	64 80	29 20	14 5	Eldridge and Griffin 1983 Griffin *et al* 1987
E. grandis	- -	9 79	- 0	- 100	- 0	 11	Hodgson 1976a Hodgson 1976b
E. ovata	84	73	0	86	14	7	Potts unpubl. data
E. nitens	77 -	72 74	14 1 0	86 100	0 0	14 4	Tibbits 1988 *APPM Forest Products*
E. globulus	74	75	8	77	15	13	Potts unpubl. data
E. gunnii	81	73	20	60	20	10	Potts and Cauvin 1988
E. morrisbyi	100	-	100 84	0 11	0 5	4 19	from Table 2 self (bagged + flies) c.f. O.P.
E. urnigera	-	57	0	100	0	2	Tibbits unpubl. data
E. pulverulenta	-	-	30-40	40-50	20	10	Pryor 1976

Table 2. Seed yield in a 4 x 4 diallel mating of *E. morrisbyi*.

The table indicates the number of full seed obtained per flower crossed in 1986 and 1987 (lower). Results are shown from both assisted and unassisted (italics) self-pollination and open-pollination (O.P.). The average number of flowers per cross is fifty-nine (range 18 - 194). ([1] branch died; [2] two bags/cross [3] three bags/cross.)

Female \ Male	MOR1	MOR2	MOR3	MOR5	O.P.
MOR1	0.0 0.0[1] *0.05* [2]	6.9 -	3.7 59.7	6.9 21.5	- 0.5 [2]
MOR2	8.2 18.9 [3]	0.0 *0.0* 0.0[3] *0.0* [2]	0.4 21.6 [2]	0.0 24.4 [3]	0.6 1.7 [2]
MOR3	1.7 24.4	3.7 -	0.0 *0.0* 0.14 *0.05* [2]	0.3 14.2	0.9 1.5[2]
MOR5	1.9 15.7	14.5 -	0.0 21.1	0.0[1] *0.0* 0.0 *0.0* [2]	0.1 0.1 [2]